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Chemical composition of some Lichen species occurring in the Namib Desert, South West Africa

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ABSTRACT

The protein content of three species of lichens from the Namib Desert was determined by means of the conventional Kjeldahl technique. Of these, *Parmelia hottentotta* had the highest protein content (16,24%), followed by *Omphalodium convolutum* (14,42%) and *Teloschistes capensis* (13,45%). Acid hydrolysis of thalli of the three species yielded fructose, galactose and glucose as well as the amino sugar glucosamine. The hydrolysate also contained a variety of amino acids.

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UITTREKSEL

Die proteininhoud van drie ligeenspesies uit die Namib-woestyn is deur middel van die konvensionele Kjeldahl-metode bepaal. Van die drie het *Parmelia hottentotta* die hoogste proteien-inhoud gehad (16,24%), gevolg deur *Omphalodium convolutum* (14,42%) en *Teloschistes capensis* (13,45%). Suurhidrolise van die drie spesies het fruktose, galaktose, glukose en die aminosuiker glukosamien opgelewer. Die hidrolisaat het ook 'n verskeidenheid aminosure bevat.

INTRODUCTION

A considerable amount of work has been published on the chemical composition of the extracellular products of lichens. The chemistry, structure, properties and distribution of these so-called lichen substances have been treated in detail by Asahina & Shibata (1954), Culberson (1969, 1970) and Culberson, Culberson & Johnson (1977). Several studies have also appeared on the chemistry of carbohydrates, free amino acids and other intracellular products. Reference to these products has been made by Huneck (1973), Mosbach (1973) and Hale (1974).

Huneck & Follmann (1971) reported on the occurrence of lichen substances in *Teloschistes capensis* (L.f.) Malme (salazinic acid, parietin) and *Omphalodium convolutum* Hue (norstictic acid, stictic acid, (+) – usnic acid). The findings of these and other authors regarding the occurrence of lichen products in other species indigenous to South West Africa are listed by Culberson (1970) and Culberson *et al* (1977).

Considerable lichen growth occurs in certain parts of the central and northern Namib Desert. More information on their chemical composition seemed of interest in view of their role as primary producers of organic matter in such an arid environment. This paper represents an effort to determine the total nitrogen content as well as the amino acid and carbohydrate composition of three lichen species from the Namib Desert.

MATERIALS AND METHODS

Lichen material

All the lichens used in this investigation were obtained through the courtesy of the Director, SWA Administration, Department of Nature Conservation and Tourism, and collected by Mr. J. Jankowitz. *Parmelia hottentotta* Ach. (Plate 1) was found approximately 35 km east of Henties Bay. *Omphalodium convolutum* (Plate 2) was collected along the gravel road between Swakopmund and Windhoek, approximately 15 km from Swakopmund. *Teloschistes capensis* (Plate 3) was collected on the gravel flats approximately 3 km north of Wlotzka's Baken.

The lichen material was thoroughly cleaned of adhering soil and rock particles.

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Plate 1: *Parmelia hottentotta* Ach.

In its natural surrounding. (Photo W. Giess)

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Plate 2: *Omphalodium convolutum* Hue.

In its natural surrounding. (Photo W. Giess)

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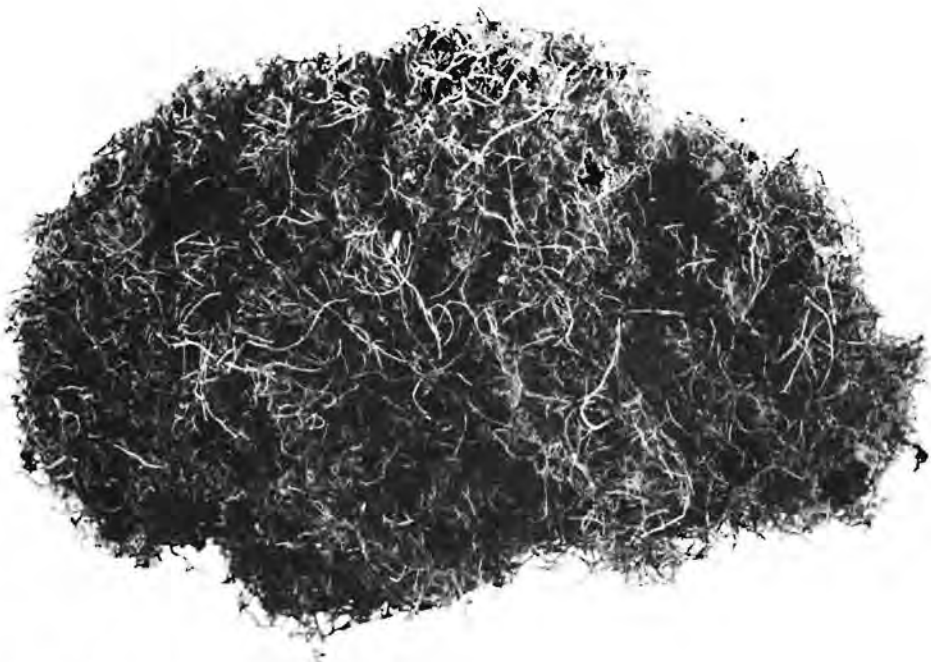

Plate 3: *Teloschistes capensis* (L.f.) Malme.

In its natural surrounding. (Photo W. Giess)

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Amino acid and amino sugar assay

The fragmented thalli were washed in 0,5 M sodium phosphate buffer (pH 7,5) and lyophilized. Ten mg of thallus material was hydrolyzed in 1 cm^3 6N HCl at 100°C for 16 h in a sealed ampule. The solution was then evaporated to dryness in a rotary flask evaporator and finally redissolved in 1 cm^3 distilled water. The pH was adjusted to pH 8,0 with 5N NaOH. The solution was centrifuged and the clear supernatant stored at 4°C. The solutions (0,1 cm^3) were diluted with 0,9 cm^3 citrate buffer (pH 2,2) and 0,2 cm^3 of this was used for assay on a Bio-Cal BC-200 automatic amino acid analyser. Resins used in the assay were of the Aminex polystyral base type with a cross-linking of 7,5 to 8,0% divinylbenzene. The assay specifications were as follows:

1. Basic amino acids

Column	– 25 cm with internal diameter of 0,9 cm
Resin	– Aminex A5, particle size 11,5–15,5 μm
Resin height	– 14 cm
Buffers	– pH 5,28; 0,35N in Na ions and 0,2N NaOH
Temperature	– 50°C

2. Neutral and acidic amino acids

Column	– 60 cm with internal diameter of 0,9 cm
Resin	– Aminex A6, particle size 15,5–19,5 μm
Resin height	– 54 cm
Buffers	– A = pH 3,25; 0,2N in Na ions B = pH 5,25; 0,2N in Na ions and 0,2N NaOH
Temperature	– 50°C

An amino acid calibration mixture supplied by Beckman Instruments was used as standard. This standard solution contained 1 cm^3 of 0,62N HCl with 2,50 ± 0,004 μmole of each amino acid. One cm^3 of this solution was diluted to five cm^3 and 0,2 cm^3 was applied to the column, giving a final concentration of 0,1 μmole. Due to the low solubility of cystine it was only present in half the concentration. The amount of each amino acid was determined by the width x height method as described in the manual. During prolonged hydrolysis with 6N HCl, the amino sugars underwent hydrolytic degradation. No attempt was made to correct for the hydrolytic loss of the amino sugars or the corresponding increases in ammonia.

Analysis of the cell sugars

A modification of the method of Cato, Cummins and Smith (1970) was used for the analysis of the cell sugars. Ten mg amounts of washed thalli were hydrolyzed in 2 cm^3 of 2N H_2SO_4 for two hours in a boiling water bath. When cooled, the acid was neutralized with solid barium hydroxide to a pH of 7,0. The precipitate was removed by centrifugation and the deposit washed with 2 cm^3 of distilled water. The supernatant was evaporated in a water bath at 60°C. The dry residue was dissolved in 0,3 cm^3 distilled water. The solution at this step was clear of any remaining barium hydroxide. One dimensional ascending thin layer chromatography was performed on silica gel G

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according to Stahl (Type 60 – Merck). The solvent system ethyl acetate (65 cm^3) and 35 cm^3 isopropanol-water mixture (2:1) was used. (Akhrem & Kuznetsova, 1965). The chromatograms were run for three hours at 5°C. Spots were visualized by spraying with acetonic-aniline-phthalate (aniline 2,0 cm^3; phthalic acid 3,0 g; acetone 95 cm^3 and 5 cm^3 water) followed by heating at 105°C for five minutes. One ul of either hydrolysate of known standard was applied to the base line of chromatogram. The relative amounts present have been arbitrarily graded as 3+, 2+, +, (+) or-, according to the size and intensity of the spot obtained.

Nitrogen Content was determined by means of the conventional Kjeldahl method. A conversion factor of 6,25 was used for calculating the crude protein content.

RESULTS AND DISCUSSION

The crude protein content of the three investigated species is shown in Table 1. In all three cases the crude protein content was unexpectedly high and compared favourably with that of lucerne hay (17,1%) and was much higher than that of veld grass hay (5,9%) (Van der Merwe, 1970)

According to Huneck (1973) the protein content of lichens varies between 1,6 and 11,4% of their dry weight. Some lichens, notably *Peltigera canina* growing in the Himalayas may, however, contain as much as 21% protein (Subramanian & Ramakrishnan, 1964). The range of amino acids found in the investigated liches was similar to those found in other plants and micro-organisms, and none of the less commonly occurring amino acids were detected. (See table 2). The highest concentration of amino acid found was glutamic acid in the case of *P. hottentotta*. There was a notable absence of cystine, hydroxy-proline and ornithine in all three species. The absence of D-, L- or meso-diaminopimelic acid is not unexpected as this amino acid has so far only been found in bacterial cell walls. Unfortunately tryptophan could not be resolved by the method used and no further attemps were made to determine the tryptophan content of the lichen thalli.

The use of the factor of 6,25 for converting Kjeldahl nitrogen into protein content, is subject to criticism, because no attempt was made to determine whether the ratio between nitrogen from protein, free amino acids, nucleic acids or chlorophyll was approximately the same as that from plant material of known composition.

The usefulness of a protein depends upon its digestibility as well as its biological value. The biological value is determined by the number and kinds of amino acids present in the molecule: The nearer the food protein approaches the body proteins in amino acid make-up, the higher will be the biological value. (McDonald, Edwards & Greenhalgh, 1973). According to data on the essential amino acid composition of proteins for non-ruminants (NRC, 1973), *O. convolutum* was low in methionine/cystine, lysine, histidine and i-leucine; *P. hottentotta* was low in histidine, while *T. capensis* was low in histidine and phenylalanine/tyrosine. Unfortunately tryptophan, which is an essential amino acid for non-ruminants, was not assayed. The uses of lichens by man as an emergency ration and as part of his daily diet has been

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discussed by Richardson (1977). As the chances are remote that the lichen growth of the area will be used as a food source by non-ruminant mammals no further consideration was given to this aspect.

In ruminant diets the amino acid composition is of less importance than the total protein and carbohydrate content. The use of lichens as a food source by vertebrates has been summarized by Richardson (1975) and Richardson & Young (1977). It was mentioned by Richardson (1975) that lichens preferred by reindeer were not those with the highest protein content but the ones which contained a high proportion of complex carbohydrates. He also stated that a deer requires the equivalent of about 2 kg of dry lichen daily. To obtain that amount an animal browses about 12 m^2 of lichen which amounts to approximately 2160 m^2 of pasture every 180 days. Because of the slow growth of lichens a particular lichen range is not ready for regrazing for 2—5 years after modest grazing. This period increases to 10—15 years after intense grazing (Richardson, 1975).

It seems that the lichen fields of the Namib Desert are utilized by animals (springbuck in particular) as a supplementary food source, especially during prolonged drought periods inland (sightings by D.C.J.W.; Coetzee[1], 1978 and Loutit[2], 1979 — personal communications). The existence of such an association will hardly be surprising in view of the universal occurrence of such relations.

Wessels, Wessels & Holzapfel (1979) reported on the relationship between two lichen-feeding Coleoptera species and *Teloschistes capensis* in the Namib Desert. The vast literature that exists on such relationships has been extensively reviewed by Gerson (1973) and Gerson & Seaward (1977). According to Gerson & Seaward (1977) insects, mites and mollusc constitute important components of the terrestrial fauna and are the main lichen grazers while the Protozoa, Rotifera and Tardigrada belong to the aquatic lichen-feeding fauna. The report of Wessels *et al* (1979) is unique in the sense that it is the first report on the existence of such an association between members of the terrestrial fauna and lichens from an arid region.

As no tests for digestibility were carried out nor the true protein or carbohydrate content determined, conclusions can not be drawn regarding the usefulness of these lichen species as a food source for animals. In view of the relationships that exist between lichens and animals of the Namib Desert, it is intended to pursue this aspect further, concentrating on insect-lichen relationships as research has already been completed.

Other low-molecular compounds, such as carbohydrates are reported to be abundant in lichens (Aspinall, Hirst & Warburton, 1955; Lindberg, Misiorny & Wachtmeister, 1953). The carbohydrates and amino sugars that could be detected in the three investigated species are shown in Table 3. No attempt was made to investigate the occurrence of polyols or polyol glycosides for which lichens are noted. Glucose, fructose and

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galactose are known constituents of lichens after chemical hydrolysis. Doubtful amounts of lactose could only be detected in *O. convolutum*. Galactosamine is a known constituent of many polysaccharides, yet was not detected in any of the three specimens analysed. Ribose was included as standard but contrary to expectations it was not detected. This can only be explained in that the concentration may have been too low to be detected.

No attempt was made to investigate the occurrence of extracellular lichen substances. These substances are mostly weak phenolic compounds and their physiological role has been the subject of considerable speculation. Huneck (1973) and Mosbach (1973) respectively discussed the nature and biosynthesis of such extracellular substances while reference to their economic uses and possible ecological role was made by Richardson (1975) and Vartia (1973).

TABLE 1: Crude protein content of three species of Namib Desert Lichens.

Species	% crude protein
Omphalodium convolutum	14,42
Parmelia hottentotta	16,24
Teloschistes capensis	13,45

TABLE 2: Quantitative amino acid analysis of three species of Namib Desert Lichens expressed as µmol per 10 mg of dried material.

Amino Acid	*Omphalodium convolutum*	*Parmelia hottentotta*	*Teloschistes capensis*
Alanine	1,50	3,15	2,20
Arginine	0,25	0,90	0,55
Aspartic Acid	1,50	3,20	2,35
Cystine	0,0	0,0	0,0
Diaminopimelic Acid	0,0	0,0	0,0
Glutamic Acid	1,50	4,60	3,75
Glycine	1,50	2,95	2,10
Histidine	0,08	0,0	0,0
Hydroxyproline	0,0	0,0	0,0
Isoleucine	0,30	0,85	0,50
Leucine	0,75	2,20	1,25
Lysine	0,35	1,66	1,10
Methionine	0,25	0,50	0,40
Ornithine	0,0	0,0	0,0
Phenylalanine	1,60	1,45	0,0
Proline	1,40	2,35	1,55
Serine	1,25	2,40	1,80
Threonine	1,30	2,15	1,45
Tryptophan	—	—	—
Tyrosine	0,0	0,90	0,0
Valine	0,55	1,25	0,95
Ammonia	2,60	4,30	3,60

— = Not assayed

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TABLE 3: Qualitative amino sugar and carbohydrate analysis of three species of Namib Desert Lichens.

Amino sugars and carbohydrates	*Omphalodium convolutum*	*Parmelia hottentotta*	*Teloschistes capensis*
Glucosamine	+	+	+
Galactosamine	–	–	–
Monosaccharides			
Arabinose	–	–	–
Fructose	++	+++	++
Glucose	+	–	(+)
Mannose	–	–	–
Rhamnose	–	–	–
Galactose	–	+	–
Disaccharides			
Lactose	–	(+)	–
Maltose	–	–	–

+ present in small amounts; ++ present in intermediate amounts; +++ present in appreciable amounts; (+) doubtful; – absent.

SUMMARY

The thalli of three lichen species collected from the Namib Desert (*Omphalodium convolutum*, *Parmelia hottentotta* and *Teloschistes capensis*) were analysed for their crude protein content, amino acid composition of the proteins and the sugar composition of the carbohydrates.

The crude protein content, calculated from the total nitrogen content as determined by means of the conventional Kjeldahl method, was found to be unexpectedly high in all three cases (14,42% for *O. convolutum*, 16,24% for *P. hottentotta* and 13,45% for the *T. capensis*.) The amino acid composition of the thallus proteins was determined by means of an automatic amino acid analyser after acid hydrolysis. The range of amino acids found was similar to those found in higher plants and micro-organisms, and none of the less commonly occurring amino acids was detected. The highest concentration of amino acid found was that of glutamate in *P. hottentotta*. There was a notable absence in all three cases of cystine, hydroxy-proline and ornithine. The digestibility of the thalli by ruminants as well as non-ruminants was not determined accordingly, no conclusions could be drawn regarding the usefulness of the lichen thalli as a food source.

Carbohydrates were analysed by acid hydrolysis and thin layer chromatography. Fructose and glucosamine were detected in all three species, the former in appreciable amounts. Glucose was detected in only two of the three cases, whereas galactose and lactose were found in only one species.

Reference has also been made to the role of lichens as a food source for animals in the area.

Plates of the three lichen species are presented.

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